JOURNAL OF AEROSOL MEDICINE
Volume 9, Number 1, 1996
Mary Ann Liebert, Inc.

Liposomal Aerosols in the Management of Pulmonary Infections

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ABSTRACT

The combination of liposomes and aerosols has been utilized to directly target the lungs with chemotherapeutic agents that might not have been used because of low solubility or toxicity. There are a variety of antibacterials, antifungals, and antivirals that have good *in vitro* activity, but are not effective because of their systemic toxicity and/or poor penetration into the lungs. Incorporation of many lipophilic drugs into liposomes decreases their toxicity without affecting effectiveness, thus increasing the therapeutic index. We have focused on aerosol delivery of amphotericin B (ampB) for the treatment of pulmonary and systemic fungal diseases. We have tested a variety of ampB-lipid formulations for the optimal treatment regimen for *Cryptococcus* and *Candida* infections in mouse models. The AeroTech II nebulizer (MMADs of 1.8–2.2 μm) produced aerosols with the highest concentrations in the breathable range. Pharmacokinetic studies revealed that pulmonary drug was present for hours to weeks. AmBisome retained its anticryptococcal activity even when animals were challenged 14 days after aerosol treatment. Aerosols may also be effective in systemic diseases. In our *Candida*–mouse model, systemic candidiasis and mortality were reduced by aerosolized ampB–liposome treatment. The ability to utilize lipophilic drugs, to deliver high concentrations of drug directly to the site of infection, and to reduce toxicity makes aerosol liposomes an attractive, alternative route of administration.

Key words: aerosol, liposomes, pulmonary diseases, amphotericin B, treatment

INTRODUCTION

PULMONARY INFECTIONS with a variety of pathogens, especially in immunocompromised individuals, are important causes of morbidity and mortality. Despite the availability

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of better antibiotics and antivirals, treatment failures are not uncommon. In the search for more effective chemotherapeutic agents, we decided to reevaluate some compounds that were very effective, but that suffered from solubility, toxicity, and/or pulmonary localization (targeting) problems. For respiratory infections, we began to solubilize chemotherapeutic agents by incorporation into liposomes and targeting the drug–liposome formulations directly to the lungs by aerosol administration. In many instances, incorporation of lipophilic drugs into liposomes decreases their toxicity without affecting effectiveness. Aerosolization of these agents may produce 10- to 100-fold greater lung concentrations when administered in similar or usually lower mg/kg/day doses than by other more conventional routes. Thus, this form of drug delivery may increase local effectiveness while decreasing systemic toxicity, creating a larger therapeutic window.

In the present report, examples of liposomal drugs for aerosol treatment of viral, bacterial, and mycotic infections of the lungs will be given (Table 1). The major focus will be on aerosol delivery of amphotericin B-liposomes for the treatment of fungal infections. Pulmonary targeting has been used to achieve high local concentrations of drug in respiratory secretions since systemic treatment for infectious diseases of the lungs has not been very effective (for a review of systemic use of liposomal drugs for infectious diseases, see Bakkerwoudenberg et al., 1994; Wasan and Lopezberestein, 1995). In addition, aerosol administration of some drug–liposome formulations may be an avenue for effective systemic treatment.

TABLE 1. POSSIBLE AREAS FOR AEROSOL LIPOSOME TREATMENT OR PREVENTION OF PULMONARY INFECTIONS

Areas for current or future use	*Liposomal drug for aerosol treatment*
Bacterial pneumonia	
Streptococcus pneumoniae	Ceftazidime
Klebsiella pneumoniae	Kanamycin, gentamicin,
Pseudomonas aeruginosa	Amikacin, tobramycin
Mycobacterial infections	
M. avium-intracellulare complex	Rifamycin, ethambutol, pyrazinamide, ceftazidime,
M. tuberculosis	ethionamide, isoniazid, clofazimine, etc.
Viral infections	
Rhinovirus	Enviroxime
	WIN compounds
Viral pneumonia	
CMV	Nucleoside-lipid analogs
Influenza/parainfluenza viruses	
Adenovirus	
HIV	AZT-lipid analog
	Amphotericin B/nystatin
Fungal pneumonia	
Aspergillus	Amphotericin B
Systemic fungi	
Pneumocystis carinii	
Vaccines and immunomodulators	
Mucosal immunity (viruses/bacteria)	Peptides (MDP, T/B cell epitopes)
	Interleukins
Antioxidants	Superoxide dismutase

LIPOSOMAL DRUGS FOR AEROSOL TREATMENT OF BACTERIAL INFECTIONS OF THE LUNGS

Secondary bacterial pneumonia is an important cause of morbidity and mortality, especially in elderly individuals. These suppurative lung diseases can be difficult to treat and may result in a persistent parenchymal inflammation, and destruction (Tag El-Din et al., 1994). In addition, nosocomial pneumonia is reported to occur in 9 to 60% of mechanically ventilated patients hospitalized in intensive care units and with mortality rates of 20 to 50%. While a variety of antibiotics are available, many useful ones have toxic side effects and/or do not penetrate the lungs well. Thus, the treatment of respiratory bacterial infections with aerosolized antibiotics should be advantageous, as this modality achieves higher levels in respiratory secretions, has a lower incidence of systemic side effects, and has an earlier onset of action than oral or intravenous therapy (Tag El-Din et al., 1994; Baldwin et al., 1992).

Aerosol administration of antimicrobial compounds has been tested in animal models for 53 years (Barach et al., 1942). An early study administered the aminoglycoside kanamycin as an effective treatment of respiratory *Klebsiella pneumoniae* infection in mice (Berendt et al., 1975). More recently, streptomycin and ciprofloxacin entrapped in liposomes were found to be less toxic and more effective than free drug when administered systemically for the treatment of experimental salmonellosis in mice (Tadakuma et al., 1985; Magallanes et al., 1993). In cystic fibrosis patients, aerosolized tobramycin has been used in an attempt to directly target the lungs and treat this severe disease (Leconte et al., 1993; Fiel, 1995). Also, endotracheal and aerosol administration of ceftazidime, and of free and liposome-encapsulated tobramycin in animal models has demonstrated the advantages of aerosol delivery, more direct and longer residence time within the lungs (Omri et al., 1994; Bressolle et al., 1992; Makhoul et al., 1993).

With the increase in mycobacterial diseases [drug-resistant *Mycobacterium tuberculosis* and *M. avium-intracellulare* complex (MAC)] in recent years, there has been an increased interest in better treatment modalities. Antimycobacterial activity of streptomycin and ciprofloxacin, and rifampin was shown to be enhanced when these drugs were delivered to macrophages in a liposome-encapsulated form (Majumdar et al., 1992; Agarwal et al., 1994). In AIDS patients, intravenous liposome-encapsulated gentamicin treatment of MAC reduced blood colony counts by 75% and was well tolerated (Nightingale et al., 1993).

Our laboratory has been interested in utilizing liposomal aerosol treatment with combinational antimycobacterial drugs. We have been able to formulate a variety of antimycobacterial drugs (e.g., ethambutol, pyrazinamide, capreomycin, ciprofloxicin, ethionamide, isoniazid) into dilauroylphosphatidylcholine (DLPC)–liposomes. Aerosolization of these formulations with the AeroTech II nebulizer at a flow rate of 10 liters/min produced particles in the size range of 1–2 μm. In addition, particle size varied between free drug and liposome-associated drug. The latter were more suited for deposition throughout the respiratory tract.

Thus, the combination of liposomal drug and aerosolization should be a very effective method for treating bacterial pneumonias.

LIPOSOMAL DRUGS FOR AEROSOL TREATMENT OF VIRAL INFECTIONS OF THE LUNGS

Administration of the antiviral drug, ribavirin, by aerosol was shown to be effective for the treatment of influenza A and B virus and respiratory syncytial virus pulmonary infec-

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tions in man (Gilbert and Knight, 1990; Knight and Gilbert, 1988; Knight et al., 1986). Although not many antiviral compounds are available for the treatment of respiratory infections, the few are often too insoluble in aqueous solutions and/or toxic to be effectively utilized by aerosol. To overcome these problems, we sought out drugs with very good antiviral activity whose administration would be enhanced by incorporation into liposomes and aerosolized. We began in 1985 with the antirhinovirus compound, enviroxime.

Enviroxime inhibits the *in vitro* replication of all rhinoviruses in the 10 to 100 ng/ml range. However, clinical evaluations in humans had not consistently shown efficacy, principally, we believed, due to the lack of an appropriate method for administering this very water-insoluble drug. By incorporation of enviroxime into liposomes composed of egg yolk phosphatidylcholine (EYPC), we were able to increase its solubility by more than a 1000-fold and to decrease its cytotoxicity while maintaining its antiviral activity (Gilbert et al., 1988; Knight and Gilbert, 1988; Wyde et al., 1988; Six et al., 1989). Small-particle aerosols were generated with a Puritan-Bennett nebulizer giving mass median aerodynamic diameters (MMAD) of 2.0 to 2.5 μm. In contrast to free enviroxime, which could not be delivered by aerosol because of its water insolubility, liposomal enviroxime was readily delivered to the upper and lower respiratory tracts of mice. After just 20 min, significant levels of enviroxime were detected in the lungs and noses of mice exposed to the aerosol. In addition, mice exposed to aerosols of liposomes containing both enviroxime and fluorescein isothiophosphatidylethanolamine showed accumulations of the fluorescent marker in the lungs, in or around the tall columnar epithelial cells lining the bronchi and bronchioles. Recently in an aerosol study with normal volunteers, ^{99m}Tc-labeled DLPC liposomes showed very good pulmonary distribution, which was dependent on the particle size produced by the nebulizer (Vidgren et al., 1995). These studies indicated that liposome aerosols offered a method for the delivery of hydrophobic compounds for the treatment of respiratory diseases. During the ensuing years, a series of potentially useful antirhinovirus compounds, the "WIN" drugs, have demonstrated excellent activity *in vitro,* but have had marginal success in human studies (Andries et al., 1991; Turner et al., 1993; Sperber and Hayden, 1988). These compounds are very insoluble in water and would benefit from incorporation into liposomes and direct aerosol delivery to the infected respiratory tract.

Currently, there is a variety of viral infections that cause pneumonia, especially in the immunocompromised individual, such as cytomegalovirus (CMV), influenza viruses, adenoviruses, and human immunodeficiency virus (HIV). Of the antivirals that are available, licensed or experimental, none has been very effective mainly due to toxicity. Targeted pulmonary delivery of an antiviral with reduced toxicity and prolonged clearance might be beneficial. Aerosolized liposomal formulations could provide these characteristics. The technology is available to add lipophilic derivatives to drugs, especially the nucleoside analogs, which would allow their association with liposomes and delivery by aerosol (Hostetler et al., 1993, 1994; Schwendener et al., 1994; Sidwell et al., 1995).

LIPOSOMAL VACCINES AND IMMUNOMODULATORS FOR AEROSOL TREATMENT OF INFECTIONS OF THE LUNGS

Protection from viral and bacterial infections by vaccination is not a new concept. However, in recent years, the concept that direct application of an antigen to the natural

site of infection will lead to a more effective and longer lasting immunization has been put forth. Encapsulation of antigens and/or immunomodulators such as interleukin-2 (IL-2) into liposomes has been an effective method for antigen presentation and stimulation of the immune response (Mbawuike et al., 1995; Vanrooijen, 1990; Garcon and Six, 1991; El Guink et al., 1989; Brynestad et al., 1990; Anderson et al., 1992, 1994; Kedar et al., 1994).

We believe that aerosol delivery of vaccine and/or immunomodulators will be an easy and effective way to develop local pulmonary mucosal immunity (IgA antibodies in the respiratory secretions of the upper respiratory tract), as well as systemic immunity and IgG antibodies in the respiratory secretions of the lower respiratory tract (Waldman et al., 1973).

LIPOSOMAL ANTIOXIDANTS FOR AEROSOL TREATMENT OF INFECTIONS OF THE LUNGS

Antioxidants such as superoxide dismutase (SOD) have been studied for the prevention of lung disease or for affecting the consequences of infection. For example, as part of the pathogenesis of influenza virus infection, there is an overreaction of the immune responses of the host as well as a direct effect of virus multiplication (Wilson et al., 1980; Wyde et al., 1977; Couch and Kasel, 1983; Hennet et al., 1992). When SOD was conjugated with a pyran copolymer, mice were protected from lethal influenza virus infection when administered 5 to 8 days after infection (Oda et al., 1989). We have found also that aerosolized SOD in RSV-infected cotton rats reduced virus lung titers (unpublished data). SOD has been incorporated into liposomes and retains its biological activity (Walther et al., 1993; Welsh et al., 1994; Stanimirovic et al., 1994; Briscoe et al., 1995). *In vitro* studies and systemic administration in animal models have demonstrated the advantages of liposomal incorporation. For pulmonary delivery, SOD aerosols will be advantageous (Gillissen et al., 1993; Shek et al., 1994) and possible interactions with surfactant protein(s) may enhance the delivery of liposomal antioxidants to lung cells (Walther et al., 1993; Davis et al., 1994).

LIPOSOMAL DRUGS FOR AEROSOL TREATMENT OF FUNGAL INFECTIONS OF THE LUNGS

The incidence of progressive pulmonary and disseminated forms of cryptococcosis, histoplasmosis, and coccidioidomycosis all have increased in AIDS patients [reviewed in Diamond (1991)] while immunosuppression of transplant patients has been a major cause of pulmonary and systemic fungal infections, especially with *Aspergillus*. Treatment of these infections has been less successful than other systemic fungal infections.

Amphotericin B (ampB) has been effective in the treatment of systemic fungal infections, although this antifungal agent is often quite toxic. While incorporation of ampB into liposomes reduces the drug's cytotoxicity (Brajtburg et al., 1990; Lopez-Berestein et al., 1983; Lopez-Berestein, 1988), it does not diminish the antifungal activity. However, cardiopulmonary toxicity and anaphylactic reactions after liposomal ampB infusions have been reported (Levine et al., 1991; Laing et al., 1994; Aguado et al., 1993). Systemic treatment with ampB–liposomes leads to the rapid clearance of the liposomes by the reticulo-

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endothelial system. Thus, higher doses of drug are required to obtain sufficient quantities of drug in the lungs.

To better administer ampB to the lungs, the combined use of aerosolization to specifically target the lungs and liposomal ampB to reduce toxicity has been used. In murine models of *Cryptococcus* and *Candida,* the efficacy of ampB–liposomes when administered as an aerosol in the treatment of both pulmonary and systemic diseases has been demonstrated (Gilbert et al., 1992, 1994). In animal models of aspergillosis, prophylactically administered aerosolized ampB was effective in reducing mortality (Niki et al., 1991; Allen et al., 1994).

We have evaluated jet type nebulizers and liposome formulations to achieve optimal delivery of ampB to the lungs. Initially, amphotericin B formulated in liposomes of EYPC was used in the aerosol treatment of mice infected intranasally with *Cryptococcus neoformans* or intravenously with *Candida albicans* (Gilbert et al., 1992, 1994). Aerosol administration of liposomal ampB was more effective than intravenous in reducing *Cryptococcus* colonization of the lungs and increased the duration of survival of infected animals. With systemic *Candida* infection, aerosol delivery of liposomal ampB reduced the numbers of organisms in the kidneys and increased the survival time. These studies indicated that aerosol administration could treat both local, pulmonary, and systemic infections.

AmBisome, a commercially available ampB–liposome preparation (Proffitt et al., 1991), has been previously shown to be more effective than conventional deoxycholate amphotericin B in the treatment of pulmonary aspergillosis in persistently granulocytopenic rabbits (Francis et al., 1994), in systemic candidiasis in leucopenic mice (Vanetten et al., 1993), and in other fungal infections (Clemons and Stevens, 1991, 1993). In man, intravenous AmBisome has been given to immunosuppressed bone marrow transplant patients with a reduction in toxicity and some beneficial effects (Guillemain et al., 1994; Ringden et al., 1994). Thus in the optimization of the ampB–liposome aerosolization system, AmBisome and the AeroTech II nebulizer, which is commercially available, have been used to study pharmacokinetics, and prophylactic and therapeutic efficacy in a *Cryptococcus*–mouse model following aerosol administration.

A pharmacokinetic study of aerosolized AmBisome (4 mg ampB/ml in the reservoir of an AeroTech II nebulizer; 0.6 mg ampB/kg/20 min exposure) demonstrated that just 3 aerosol treatments with AmBisome every other day was sufficient to attain lung levels well above any inhibitory concentration for fungi and that even after 14 days postexposure; there was sufficient drug present in the lungs to eliminate *Cryptococcus* from the lungs. Both prophylactic and therapeutic treatment protocols were effective in reducing the number of organisms colonizing the lungs and the gross lung scores (Table 2).

CONCLUSIONS

There are many areas where aerosols of liposomal drugs could be useful in the treatment and prevention of infectious and/or immunological diseases of the lungs. Incorporation of potentially useful drugs into liposomes instead of using free drug has several advantages: solubility of lipophilic drugs allows for much greater concentrations of drug to be used; in many cases, incorporation decreases a drug's toxicity without affecting its inhibitory effects, and liposomal formulations may lead to better pharmacokinetics such

TABLE 2. EFFECTS OF AEROSOLIZED AMBISOME ON *CRYPTOCOCCUS*-INFECTED MICE

	Prophylactic[a]			*Therapeutic*[b]	
Treatment	*Control*	*Infected on day 0*	*Infected on day 14*	*Control*	*Treated 2×/week*
Cryptococcus (log_{10} CFU/lung)	4.6	0	0	4.7	0
Mean gross lung scores ($0 \rightarrow 4+$)	3.4	1.0	1.0	3.8	2.4

[a]Mice ($N = 10$/group) were exposed to aerosol of liposomes only (no drug), or AmBisome (4 mg ampB/ml; 0.6 mg/kg/day) on days −7, −5, and −3. On day 0 and +14, mice were infected intranasally with *Cryptococcus*. On day +49, lungs were scored and organisms quantitated.

[b]Mice ($N = 10$/group) were infected intranasally with *Cryptococcus* on day 0 and treated with liposomes only or AmBisome (4 mg ampB/ml) twice a week for 6 weeks starting on day +7. On day +49 (3 days after the last treatment), lungs were scored and organisms quantitated.

that shorter and/or fewer treatments are necessary. Drug-containing liposomes can be readily aerosolized with particle characteristic that target the entire respiratory tract. We believe that aerosol delivery is an effective route of administration for local treatment and, in many cases, also may be very effective for the treatment of systemic diseases.

ACKNOWLEDGMENTS

These studies were supported by funds from the Clayton Foundation for Research, 1 Riverway, Suite 1440, Houston, TX 77056.

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